Patent claims

1. A method for detecting different nucleic acids A in parallel, comprising the following steps:

a) providing in each case one first primer pair which is suitable for carrying out a PCR together with one of the nucleic acids A and which contains a first primer (P1) and a second primer (P2),

with the first primer (P1) exhibiting a 5'-terminal first constituent segment (c1) and a 3'-terminal second constituent segment (c2) and the second primer (P2) exhibiting a 5'-terminal third constituent segment (c3) and a 3'-terminal fourth constituent segment (c4),

with the sequences of the second constituent segment (c2) and the fourth constituent segment (c4) being selected such that the second constituent segment (c2) can hybridize specifically, under defined first conditions, with a predetermined first segment of the one of the nucleic acids A, and be enzymically extended, and the fourth constituent segment (c4) can hybridize specifically, under defined second conditions, with a predetermined second segment of a nucleic acid A' which is complementary to the one of the nucleic acids A, and be enzymically extended, and

with in each case an intermediate segment i, which connects the first constituent segment (c1) to the second constituent segment (c2) and is specific for the second constituent segment (c2), or an intermediate segment i, which connects the third constituent segment (c3) to the fourth constituent segment (c4) and is specific for the fourth constituent segment (c4), being provided,

with the first (P1) or second primers (P2) of the first

primer pairs in each case differing in the intermediate segment i and in the second constituent segment (c2) or fourth constituent segment (c4) which is arranged in connection thereto, with each of the second (c2) or fourth constituent segments (c4) being specific for precisely one of the nucleic acids A,

b) bringing the different nucleic acids A, or the nucleic acids A' which are complementary thereto, into contact with the first primer pairs in a solution and carrying out a first primer extension reaction in which the first primers (P1) are extended, under the first conditions, or the second primers (P2) are extended, under the second conditions, at least once and at least so far that the respective other primers (P2, P1) of the first primer pairs are able to bind specifically, under the first or second conditions which are required for their specific hybridization, to in each case one first primer extension product which is formed in this connection,

c) carrying out a second primer extension reaction in which the first primer extension products in each case serve as a template and the respective second (P2) or first primers (P1) are extended, under the first or second conditions which are required for their specific hybridization with the respective first primer extension products, with the formation of in each case one second primer extension product,

d) providing in each case one second primer pair which in each case contains a third primer (P3) and a fourth primer (P4) and which is suitable for carrying out a PCR together with the respective second primer extension products,

with the sequences of the third primer (P3) and fourth primer (P4) being in each case selected such that the

third primer (P3) can in each case hybridize specifically, under defined third conditions, with a sequence which is complementary to the first constituent segment (c1), and be enzymically extended, and the fourth primer (P4) can in each case hybridize specifically, under defined third conditions, with a sequence which is complementary to the third constituent segment (c3) and be enzymically extended,

e) bringing the second primer extension products into contact with the respective second primer pairs and carrying out a PCR, with in each case the intermediate segment i, or an intermediate segment i' which is complementary thereto, being amplified with the formation of third primer extension products,

f) providing in each case one immobilized probe (Pr) for each nucleic acid A to be detected, with the probe (Pr) being in each case able to hybridize specifically, under defined fourth conditions, with one of the intermediate segments i or one of the intermediate segments i' which are complementary thereto,

g) bringing the probes (Pr) into contact with the third primer extension products under the fourth conditions, and

h) detecting the third primer extension products which bind, or are bound, to the probes (Pr).

2. The method as claimed in claim 1, wherein the first primer extension reaction and the second primer extension reaction are carried out as PCRs.

3. The method as claimed in one of the preceding claims, wherein the first primer extension reaction and/or the second primer extension reaction and/or the PCR(s) is/are carried out under hot start conditions.

4. The method as claimed in one of the preceding claims, wherein the first primer extension reaction is carried out, under the first conditions, and/or the second primer extension reaction is carried out, under the second conditions, at most 10 times, preferably at most 5 times, in particular at most 2 times.

5. The method as claimed in one of the preceding claims, wherein the sequences of the first constituent segment (c1) and third constituent segment (c3) are selected such that the third conditions can be so stringent that the second constituent segment (c2) does not significantly hybridize, under the third conditions, with the first segment of the one of the nucleic acids A and the fourth constituent segment (c4) does not significantly hybridize, under the third conditions, with the second segment of the nucleic acid A' which is complementary to the one of the nucleic acids A.

6. The method as claimed in one of the preceding claims, wherein the sequences and concentrations of the first (P1), second (P2), third (P3) and fourth primers (P4) are selected such that the specific annealing temperatures of the third primer (P3), which hybridizes with the sequence which is complementary to the first constituent segment (c1), and of the fourth primer (P4), which hybridizes with the sequence which is complementary to the third constituent segment (c3), are in each case at least 5°C higher than the in each case higher annealing temperatures of the second constituent segment (c2), which hybridizes with the first segment of one of the nucleic acids A, and of the fourth constituent segment (c4), which hybridizes with the second segment of the complementary nucleic acid A'.

7. The method as claimed in one of the preceding claims, wherein step e is carried out in the solution.

8. The method as claimed in one of the preceding claims, wherein at least steps a to e, in particular steps a to h, are carried out in a closed vessel which is not opened between the steps.

9. The method as claimed in one of the preceding claims, wherein the concentration, in the solution, of the first or second primer (P1, P2) containing the intermediate segment i is selected to be so low that this primer (P1, P2) does not significantly inhibit a hybridization of the probe (Pr) with the respective intermediate segment i, or the intermediate segment i' which is complementary thereto, of the third primer extension products in step g.

10. The method as claimed in one of the preceding claims, wherein the concentration, in the solution, of the in each case first primer pair is set to be from 0.001 to 0.1 μmol/l.

11. The method as claimed in one of the preceding claims, wherein the ratio of the concentrations of the in each case first primer pair to the in each case second primer pair is less than 1:10, preferably less than 1:100, particularly preferably less than 1:1000.

12. The method as claimed in one of the preceding claims, wherein the second primer pairs are added to the solution prior to the first primer extension reaction.

13. The method as claimed in one of the preceding claims, wherein, in step e, in each case the third primer (P3) or the fourth primer (P4) is extended more frequently than is the respective other primer (P4, P3)

of the respective second primer pairs.

14. The method as claimed in one of the preceding claims, wherein, in the second primer pair which is provided in step d, the third primer (P3) or the fourth primer (P4) is present in excess as compared with the respective other primer (P4, P3) which is present therein.

15. The method as claimed in one of the preceding claims, wherein a multiplicity of first primer pairs whose first primers (P1) exhibit an in each case identical or almost identical first constituent segment (c1) and/or whose second primers (P2) exhibit an in each case identical or almost identical third constituent segment (c3), and whose second constituent segment (c2) or fourth constituent segment (c4) is in each case specific for precisely one of the nucleic acids A, is added to the solution.

16. The method as claimed in one of the preceding claims, wherein the sequences of the first (P1), second (P2), third (P3) and fourth primers (P4) are selected such that they do not form any primer dimers and/or do not hybridize with themselves or with each other in the method.

17. The method as claimed in one of the preceding claims, wherein the sequences of the intermediate segments i are selected such that neither they themselves, nor the intermediate segments i' which are complementary thereto, hybridize, in the method, with themselves or with the first (c1), second (c2), third (c3) or fourth constituent segments (c4) or their complementary sequences.

18. The method as claimed in one of the preceding claims, wherein the sequences of the intermediate

segments i are selected such that hybrids of the intermediate segments i with nucleic acids which were in each case completely complementary thereto would have melting temperatures which are essentially identical, lying, in particular, in a temperature range of 5°C.

19. The method as claimed in one of the preceding claims, wherein, for specifically detecting one of the nucleic acids A in the presence of another nucleic acid which only differs from the one of the nucleic acids A in one first base which is contained in the one of the nucleic acids A, the sequences of the first (P1) or second primers (P2) are selected such that the respective base of the second (c2) or fourth constituent segment (c4), which base is complementary to the first base or to a second base, which is complementary thereto, of a complementary nucleic acid A', is located at the 3' end, or in the vicinity of the 3' end, of the in each case first (P1) or second primer (P2).

20. The method as claimed in one of the preceding claims, wherein the second (c2) or fourth constituent segments (c4) contain a base which is not complementary to a third base, which corresponds to it in its position, in the first segment of the one of the nucleic acids A or in the second segment of the nucleic acid A'.

21. The method as claimed in one of the preceding claims, wherein the respective sequences of the first (P1), second (P2), third (P3) and fourth primers (P4), and of the probe (Pr), are selected such that in each case the first, in each case the second, in each case the third and/or in each case the fourth conditions for detecting the different nucleic acids A are identical.

22. The method as claimed in one of the preceding claims, wherein the probe (Pr) is in each case immobilized on an electrode (E) or in its immediate vicinity.

23. The method as claimed in one of the preceding claims, wherein the detection in step h is effected by detecting a change in a fluorescence-optical property or a change, which is determined by the hybridization, in an electrical property at the electrode (E).

24. The method as claimed in claim 23, wherein a change in a redox property, in particular in association with the oxidation of guanine or adenine residues of the third primer extension products, in an impedance or in a conductivity is measured, as the change in the electrical property, using the electrode (E).

25. The method as claimed in one of the preceding claims, wherein the third primer (P3) and/or the fourth primer (P4) exhibits a label which can be detected, in particular, fluorescence-optically or electrically or electrochemically by means of the electrode (E) and which is preferably redox-active.

26. The method as claimed in claim 25, wherein the label exhibits a specific affinity molecule, an osmium complex, a nanogold particle, a cysteine, ferrocenyl, daunomycin, benzoquinone, naphthoquinone, anthraquinone or p-aminophenol group, a dye, in particular indophenol, thiazine or phenazine, or a fluorescent dye, in particular 6-FAM, HEX, TET, Cy3, Cy5, IRDye™700, IRDye™800, Biodipy, fluorescein, Joe, Rox, TAMRA or Texas Red.

27. The method as claimed in claim 25 or 26, wherein the label is an affinity molecule and it is detected

using a counter molecule which specifically binds the affinity molecule, with the counter molecule being conjugated with an enzyme which can convert a substrate such that the reaction product can be specifically detected electrochemically or optically.

28. The method as claimed in one of the preceding claims, wherein use is made of a multiplicity of different probes (Pr) which are complementary to the intermediate segments i or to the intermediate segments i' which are complementary thereto, each of which probes is bound to, or in the immediate vicinity of, a separate electrode (E).

29. The method as claimed in one of the preceding claims, wherein use is made of a multiplicity of electrodes (E) which are arranged on a surface, in particular an electrode chip, so as to be individually bonded or bondable.

30. The method as claimed in one of the preceding claims, wherein an RNA is detected indirectly by transcribing it into a DNA and then detecting the DNA as nucleic acid A.

31. A kit for carrying out a method, as claimed in one of the preceding claims, for detecting a multiplicity of different nucleic acids A in parallel, with the kit comprising:

a) for each nucleic acid A to be detected, in each case one first primer pair which is suitable for carrying out a PCR together with the nucleic acid A and which contains a first primer (P1) and a second primer (P2),

with the first primer (P1) exhibiting a 5'-terminal first constituent segment (c1) and a 3'-terminal second

constituent segment (c2) and the second primer (P2) exhibiting a 5'-terminal third constituent segment (c3) and a 3'-terminal fourth constituent segment (c4),

with the sequences of the second constituent segment (c2) and the fourth constituent segment (c4) being selected such that the second constituent segment (c2) can hybridize specifically, under defined first conditions, with a predetermined first segment of the nucleic acid A which is in each case to be detected, and the fourth constituent segment (c4) can hybridize specifically, under defined second conditions, with a predetermined second segment of a nucleic acid A' which is complementary to the nucleic acid A which is in each case to be detected, and

with an intermediate segment i, which connects the first constituent segment (c1) to the second constituent segment (c2) and is specific for the second constituent segment (c2), or an intermediate segment i, which connects the third constituent segment (c3) to the fourth constituent segment (c4) and is specific for the fourth constituent segment (c4), being provided, and

b) for each nucleic acid A to be detected, in each case a second primer pair containing a third primer (P3) and a fourth primer (P4), which pair is suitable, together with a primer extension product which can be generated, using the first (P1) and second (P2) primers, when the nucleic acid A which is in each case to be detected is present, for carrying out a PCR, and

with the sequences of the third primer (P3) and fourth primer (P4) being selected such that the third primer (P3) can hybridize specifically, under defined third conditions, with a sequence which is complementary to the first constituent segment (c1) of the first primer,

and the fourth primer (P4) can hybridize specifically, under defined third conditions, with a sequence which is complementary to the third constituent segment (c3) of the second primer.

32. The kit as claimed in claim 31, which in each case contains, for each nucleic acid A to be detected, a probe (Pr) which can in each case hybridize specifically, under defined fourth conditions, with the intermediate segment i or the intermediate segment i' which is complementary thereto.

33. The kit as claimed in claim 32, wherein the probes (Pr) are immobilized.

34. The kit as claimed in one of claims 31 to 33, wherein the first constituent segments (c1) of the first primers (P1) contained in the kit are identical and/or the third constituent segments (c3) of the second primers (P2) contained in the kit are identical.

35. The kit as claimed in one of claims 31 to 34, wherein the sequences of the intermediate segments i are selected such that the fourth conditions are identical for all the intermediate segments i or the intermediate segments i' which are complementary thereto.

36. The kit as claimed in one of claims 31 to 35, which contains an arrangement of electrodes (E), with in each case one probe (Pr) being immobilized on, or in the immediate vicinity of, each electrode (E) in the arrangement.

37. The kit as claimed in claim 36, wherein the arrangement of electrodes (E) is an electrode chip.

38. The kit as claimed in one of claims 31 to 37 which

contains, instead of the first primer pair, specifications for the sequences of the first constituent segment (c1), the third constituent segment (c3) and the intermediate segment i or for the sequences which are in each case complementary thereto.